Process Biochemistry 50 (2015) 941–947

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Short communication

Ketoisomeric conversion of glucose derived from microalgal biomasses

Da Gyung Lee [a], Doo Jin Choi [b], Jae Kweon Park [a,*]

[a] Department of Life Sciences, Gachon University, Seongnamdaero 1342, Seongnam-si, Gyeonggi-do 461-701, South Korea
[b] Department of Biotechnology, The Catholic University of Korea, Bucheon 420-743, South Korea

ARTICLE INFO

Article history:
Received 12 December 2014
Received in revised form 16 March 2015
Accepted 17 March 2015
Available online 26 March 2015

Keywords:
Glucose isomerase
Glucose
Fructose
Isomers
Enzymatic conversion

ABSTRACT

The purpose of this study is to elucidate the isomeric conversion of glucose to fructose using defatted microalga *Dunaliella tertiolecta* and *Botryococcus braunii*. Optimal temperature for immobilized-GI (Imm-GI) activity was determined to be 60 °C. Under optimal conditions, glucose liberated from the defatted microalgal biomasses was ketoisomerically converted to fructose in time dependent manner, identified by high performance anion-exchange chromatography equipped with a pulsed electrochemical detector (HPAEC-PED). The specific activity of Imm-GI toward sugars of microalgal biomasses was determined to be 0.69 mmol/mg for *B. braunii* and 0.83 mmol/mg for *D. tertiolecta*, respectively. The calculated value for Imm-GI for the apparent equilibrium constant, $K'_{eq} = 0.96 \pm 0.03$, at pH 6.0 and at optimum temperature 60 °C. Collectively, our proposed approach first demonstrates the contributions and potential applications of Imm-GI for the isomeric conversion of glucose liberated from defatted microalgal biomasses as a major monosaccharide to fructose.

1. Introduction

Fructose, found in many plants, vegetables and fruits, is the most water-soluble sugar. It is added to foods and drinks in the food industry for taste enhancement because of its cheaper cost and sweetness, as well as lower calories. Similarly, glucose is acquired from most of organisms including plants and fruits. Glucose and fructose are simple monosaccharides that share the same formula ($C_6H_{12}O_6$), but have different structure as isomer. For decades, many researchers have attempted to develop techniques for more efficient use of glucose. Glucose can be converted to fructose using the enzyme Glucose Isomerase (GI, EC 5.3.1.5), which can be obtained from various microbial resources such as *Clostridium thermosulfurogenes* [1], *Streptomyces violaceoniger* [2] and *Thermus aquaticus* [3]. As described above, Fructose is useful to make higher industrial value products such as high fructose corn syrup (HFCS) [4–6]. Since fructose absorption in the intestine is less than glucose [7], it is widely used in the food industry as healthier alternative for glucose [8]. Therefore the isomeric conversion of glucose to fructose is an important process for food manufacturing industry through enhancing the thermostability of glucose isomerase by protein engineering [9]. The process can be performed using immobilized GI (Imm-GI) from *Streptomyces* or other commercial products [10–12], as well as *Streptomyces* species. The use of several other species including *Bacillus thermoantarcticus*, *Clostridium thermosulfurogenes* and extremely thermophilic eubacterium, *Thermotoga maritima* for this purpose has been documented [13–15]. Among filamentous fungus, *Aspergillus oryzae* is the only one known for its use in the fermentation industry. Many industrial enzymes such as hexokinase, glucose-6-phosphate isomerase, fructose-bisphosphate aldolase, phospho-glyceromutase and pyruvate kinase are produced by this organism [16]. Collectively, the aforementioned organisms are commercially important as GI producers, as such GI has been the focus of food industry researchers. The immobilized enzymes have increased utility in the industry process due to feasibility of their reuse and stability allowing for improvement and better economics for fructose production [17–19]. GI is one of the critical enzymes with the ability to reversibly convert glucose to fructose *in vitro*. GI consists of a tetramer, and is known to be affected by metal ions, shown to increase its activity and stability [8]. Moreover, GI is known as Xylose isomerase, which converts D-xylose to D-xylulose *in vivo* [3,12,20]. This isomerization is a reversible reaction [21]. GI is usually used in an immobilized form in the food industry, creating a biocatalyst that can be reused repeatedly several times at minimum costs for the operation. Therefore, the enzyme has the largest market in the food industry because of its application in the production of high-fructose corn syrup (HFCS) [4].

* Corresponding author. Tel.: +82 32 820 4782; fax: +82 32 821 2734.
E-mail addresses: jkpark@gachon.ac.kr, jamyeong-2@nate.com (J.K. Park).

http://dx.doi.org/10.1016/j.procbio.2015.03.011
1359-5113/

Extracellular polysaccharides (EPS) obtainable from defatted microalgal biomasses are considered to be a renewable biomaterial for the conversion to monosaccharides used as source of bioethanol. Depending on the species, the structure and composition of monosaccharides of EPS can be highly variable. As Mishra and Jha demonstrated [22], EPS isolated from *Dunaliella salina* biomass was identified consisting of four major constituent monosaccharides: galactose, glucose, xylose and fructose [23]. However, less is known about the polysaccharide composition and monosaccharide production from other algal species. Especially, defatted algal biomasses were abundantly produced as uncharacterized bioresources in the process of biodiesel production. In this study we aimed explore the potential use and the production of fermentable monomeric sugar based on structural analysis of defatted biomasses based on previous reports [24], with the possibility for further enhancement of algal biomasses utilization. As demonstrated previously [24], a major component sugar of defatted microalgal biomass of *D. tertiolecta* was identified to be glucose. This is of great importance and interest to both industry and academia.

2. Materials and methods

2.1. Chemicals

Glucose isomerase (GI, EC 5.3.1.5) and Immobilized glucose isomerase (Imm-GI) from *Streptomyces murinus* used in this study were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Amount of GI needed to determine the specific activity was used according to manufacturer's instructions. Trifluoroacetic acid (TFA), 4-hydroxy-benzhydrazid (PAHBAH), monosaccharides such as glucose, fructose, xylose and glucosamine and resorcinol were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). All other chemicals used in this study were of HPLC or reagent grade.

2.2. Preparation of microalgal defatted cells

D. tertiolecta (UTEX LB 999) and *B. braunii* were purchased from the University of Texas at Austin (UTEX), since the structural analysis and utilization of biomasses were studied in the previous study [24]. Microalga were cultured in 5 L artificial sea water plus f/2 medium for 7 days at 25 °C under a continuous light intensity of 15 μE/m^2/s using white fluorescent lamps, established as the optimum culture condition with the seed culture during its exponential growing phase. Cells were collected from the culture broth by centrifugation at 10,000 rpm for 30 min and lyophilized to dry. In order to obtain defatted cells, lipids from the lyophilized cells were extracted 3 times with 10 times volume of extracting solvent consisting of chloroform and methanol (1:2, v/v), and used as crude defatted biomasses. Total amount of carbohydrates in defatted biomasses was determined by a modified phenol–sulfuric acid method [25]. Proteins were removed from the defatted dried biomass by the washing with 0.1 M NaOH and concentration of protein in the defatted biomass was determined by Bradford method [26].

2.3. Preparation of microalgal sugars

Microalgal sugars from defatted biomasses were obtained by acidic hydrolysis. Briefly, defatted biomass (0.1 g) was resuspended in 1 mL of distilled water and then mixed with an equal volume of 2.0 M TFA. Mixtures were kept for 5 h at 100 °C for acidic hydrolysis, centrifuged at 13,000 rpm for 10 min, filtered through 0.45 μm syringe filter and dried under vacuum using a Speed-Vac (Biotron, Korea). If necessary, residual acid in the hydrolysates was removed by repeated vacuum drying. The release of soluble sugars derived from hydrolysis experiments was determined using PAHBA reagent [27].

2.4. Assay of immobilized-GI activity

Preparation of activated silica-gel and immobilization of GI was performed by following the protocol as described in the previous report [28]. Herein, Seliwanoff method was adapted to quantify fructose formed after the enzyme reaction using resorcinol with slightly modified method [29]. Briefly, enzyme activity of Immobilized-GI (Imm-GI) was measured by incubating a reaction mixture consisting of 20 mM $MgSO_4 \cdot 7H_2O$ and 1 mM $CoCl_2 \cdot 6H_2O$, and mixed with 0.25 mL of Selliwanoff's reagent containing 0.1 g resorcinol and 0.1 g urea in100 mL ethanol, in the presence or absence of hydrolysates derived from defatted microalga. After that concentrated HCl was added into the mixture to be 1 N and heat for 5 min at 90 °C. Absorbance was measured at 546 nm using supernatant. In separate, fructose or glucose was prepared in ranging from 0 to 6 mg/mL to make base line for the quantitative analysis of both sugars after enzyme reaction. One unit of Imm-GI activity was defined as the amount of enzyme that produces 1 μmol of fructose from glucose per minute under the assay conditions.

2.5. Optimal pH and temperature of Imm-GI activity

The optimal pH of the Imm-GI was measured at 60 °C using buffer solutions made of different pH values, and their activities were expressed as relative activity (%) obtained at each pH. Other parameters such as temperature and stability of Imm-GI were determined under the optimum pH conditions. The following buffers (50 mM) were used: sodium acetate (pH 5.0–6.0), potassium phosphate (pH 6.0–7.0), Tris–HCl (pH 7.0–9.0), and glycine–NaOH buffer (pH 9.0–10.0). To determine the stability of the enzyme at pH values between 5.0 and 10.0, pre-incubation was performed at each pH at 4 °C for 6 days and at different temperatures for 1 h. Samples were taken at intervals and their residual enzyme activities were determined.

Optimal temperature of Imm-GI was determined at wide range of temperatures at 37 °C, 45 °C, 52 °C, 60 °C and 80 °C. Imm-GI (0.01 ± 0.001 g) was added to 0.5 mL PBS containing 20 mM $MgSO_4 \cdot 7H_2O$, 1 mM $CoCl_2 \cdot 6H_2O$ and 0.1 M glucose. After the reaction finished, the reaction was quenched by adding of 1 mL of concentrated HCl and heating for 5 min at 90 °C. After cooling down, absorbance was measured at 546 nm. Enzyme activities were expressed as relative activity (%) obtained at optimum conditions, unless described in specific activity.

2.6. Effect of ionic strength on Imm-GI activity

To determine the effects of ionic strength on Imm-GI, enzyme activity was monitored in the presence or absence of NaCl and KCl up to 1 M, respectively, as described above. Since divalent metal ions such as Ca^{2+}, Mg^{2+}, Zn^{2+} and Mn^{2+} were known to be important either inhibitors or activators of GI [8,30], effects of various metal ions (1 mM) on Imm-GI enzyme activity were assayed under optimum conditions. To determine the effect of these metal ions on Imm-GI activity, Imm-GI was treated with 5 mM EDTA at room temperature for overnight followed by dialysis against PBS (pH 7.0) with several changes of PBS. Imm-GI activity without metal ions was measured as the control. The residual activity (%) of Imm-GI was determined spectrophotometrically as described above.

2.7. HPAEC-PED analysis

The composition of monosaccharide liberated from defatted biomasses and isomeric conversion of glucose to fructose was